REMARKS

Claims 1, 2, 5-11, 13-21, 23-32, and 35-45 are presently pending. Claims 1, 5, and 10 have been amended and claim 45 added. Support for the amendments and new claim is found throughout the application as filed. The amendments of the claims have been made to emphasize features of the invention for better understanding by the examiner. The amendments and new claim raise no issue of new matter. Claims 3, 4, 12, 22, 33 and 34 have been cancelled.

Notwithstanding the foregoing, Applicants expressly reserve the right to prosecute matter no longer or not yet presented in one or more applications that may claim priority hereto. Applicants respectfully request reconsideration of the claimed invention in view of the following remarks.

Claim Objections

The objection to claim 33 as being duplicate claims has been addressed by cancellation of claim 33. New claim 45 replaces claim 33.

35 U.S.C. §112, First Paragraph

Applicants respectfully traverse the rejection of claims 1-2, 11-12, 22 and 33 under 35 U.S.C. § 112, First Paragraph, because the specification allegedly does not enable the method in conjunction with MALDI or electrospray (ESI) ionization. None of the pending claims refer to ionization using MALDI or Electrospray. Withdrawal of the rejection is respectfully urged.

35 U.S.C. §112, Second Paragraph

Applicants respectfully traverse the rejection of claim 12 as allegedly being indefinite because it refers to electrospray (ES) but depends from a claim that refers to APPI. Claim 12 has cancelled, rendering this rejection as moot.

35 U.S.C. §102 over Draisci et al.

Claims 1-3, 10, 14, 18, 21, 25, 36 and 40-42 have been rejected as allegedly being anticipated by Draisci et al. (J. Chromatography A, 2000). Claim 3 has been cancelled, rendering the rejection of this claim as moot.

The remaining claims as presently constituted (1, 2, 10, 14, 18, 25, 36 and 40-42) are not anticipated by Draisci et al., because the reference does not disclose purification of a sample by HTLC prior to tandem MS. Accordingly, withdrawal of the rejection is respectfully requested.

35 U.S.C. §102 over Tiller et al.

Claims 1-3, 10, 14, 17-18, 21, 25, 33, 36 and 40-42 have been rejected as allegedly being anticipated by Tiller et al. (J. Chromatography A, 1997). Claims 3 and 33 have been cancelled, rendering the rejection of these claims as moot.

The remaining claims as presently constituted (1, 2, 10, 14, 17-18, 21, 25, 36 and 40-42) are not anticipated by Teller et al. because the reference does not disclose purification of a sample by HTLC prior to tandem MS. Accordingly, withdrawal of the rejection is respectfully requested.

35 U.S.C. §103 over Tiller et al. in view of Merchant et al. or Robb et al., as evidenced by Breckenfeld et al.

Claims 1-3, 10-11, 13-15, 17-18, 21-25, 33-36, and 40-42 have been rejected as allegedly being obvious over Tiller et al. in view of Merchant et al. or Robb et al., as evidenced by Breckenfeld et al.

According to the Examiner, Tiller et al. teaches detection of testosterone by MS/MS/TOF which detects the specified parent and daughter ions, Merchant et al. teaches SELDI ionization with testosterone, Robb et al. teaches APPI ionization with testosterone and Breckenfeld et al. teaches various gases with MS.

Claims 3, 22, 33 and 34 have been cancelled, rendering the rejection of these claims as moot. The remaining claims as presently constituted (claims 1-2, 10-11, 13-15, 17-18, 21, 23-25, 35-36, and 40-42) are not obvious over the combination of references because none taken alone or in combination teaches the required step of purifying the sample by HTLC prior to tandem MS. Various claims are even further distinguished.

To establish a prima facie case of obviousness, three criteria must be met; there must be some motivation or suggestion, either in the cited publications or in knowledge available to one skilled in the art, to modify or combine the cited publications; there must be a reasonable expectation of success in combining the publications to achieve the claimed invention; and the publications must teach or suggest all of the claim limitations. MPEP § 2142. Thus, the Examiner has failed to establish a prima facie rejection of claims 1-2, 10-11, 13-15, 17-18, 21, 23-25, 35-36, and 40-42 over Tiller et al. in view of Merchant et al. or Robb et al., as evidenced by Breckenfeld et al., because *inter alia* this combination of references clearly does not teach or suggest all of the claim limitations. Accordingly, withdrawal of the rejection is respectfully requested.

35 U.S.C. §103 over Tiller et al. in view of Williams as evidenced by Lewis et al.

Claims 1-3, 10, 14, 17-18, 25-28, 33 and 36-43 have been rejected as allegedly being obvious over Tiller et al. in view of Williams et al. as evidenced by Lewis et al.

According to the Examiner, Tiller et al. teaches detection of testosterone by MS/MS/TOF which detects the specified parent and daughter ions, Williams et al. teaches parent and daughter ions obtained by ionizing 2, 2, 4, 6, 6-d5 testosterone, and Lewis et al. teaches summing the intensities of the most abundant ions in the MS/MS spectrum.

Claims 3, and 33 have been cancelled, rendering the rejection of these claims as moot. The remaining claims as presently constituted (claims 1, 2, 10, 14, 17-18, 25-28, and 36-43) are not obvious over the combination of references because none taken alone or in combination teaches the required step of purifying the sample by HTLC prior to tandem MS. Various claims are even further distinguished.

Thus, the Examiner has failed to establish a prima facie rejection of claims 1, 2, 10, 14, 17-18, 25-28, and 36-43 over Tiller et al. in view of Williams et al. as evidenced by Lewis et al., because *inter alia* this combination of references clearly does not teach or suggest all of the claim limitations. See MPEP § 2142. Accordingly, withdrawal of the rejection is respectfully requested.

35 U.S.C. §103 over Tiller et al. in view of Zimmer et al. in view of Quinn et al. and further in view of Starcevic et al. as evidenced by Roper-Miller et al.

Claims 1-10, 14, 17-21, 25, 33, 36, and 40-42 have been rejected as allegedly being obvious over Tiller et al. in view of Zimmer et al., in view of Quinn et al., and further in view of Starcevic et al. as evidenced by Roper-Miller et al.

According to the Examiner, Tiller et al. teaches detection of testosterone by MS/MS/TOF which detects the specified parent and daughter ions, Zimmer et al. teaches HTLC in conjunction with MS/MS for detection of protein bound drugs, Quinn et al. teaches a specified particle size for HTLC, Starcevic et al. teaches that testosterone forms highly protein bound drugs from crude plasma, and that Roper-Miller teaches deproteination techniques.

Claims 3, 4, and 33 have been cancelled, rendering the rejection of these claims as moot. Applicants respectfully traverse the rejection of the remaining claims (1-2, 5-10, 14, 17-21, 25, 36 and 40-42) as allegedly being obvious over the combination of cited references.

To establish a *prima facie* case of obviousness, three criteria must be met; there must be some motivation or suggestion, either in the cited publications or in knowledge available to one skilled in the art, to modify or combine the cited publications; there must be a reasonable expectation of success in combining the publications to achieve the claimed invention; and the publications must teach or suggest all of the claim limitations. MPEP § 2142. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *See In re Vaeck*, 947 F.2d 488, 493; 20 USPQ2d 1438, 1442 (Fed. Cir. 1991); *see* also MPEP § 2142. In analyzing obviousness, the Court of Appeals for the Federal Circuit has repeatedly cautioned that:

> [t]he factual inquiry... must be based upon objective evidence of record.... [T]he best defense against the subtle but powerful attraction of a hindsight-based obviousness analysis is rigorous application of the requirement for a showing of the teaching or motivation to combine prior art references.... [P]articular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed.

In re Sang-Su Lee, 277 F.3d 1338, 1343 (Fed. Cir. 2002), 61 USPQ2d 1430, 1433 (internal citations omitted).

The primary Tiller et al. Reference

Tiller et al. teaches use of LC-MS/MS to detect testosterone in a plasma sample. Tiller et al. quantitates testosterone from standard samples spiked into plasma using m/z ions of 97, 109 and 253 (page 123, left column). Tiller et al. shows an MS/MS chromatogram for the 4.97 min LC peak representing testosterone (Figure 3). The prominent peaks seen have m/z of 271.1, 253.1, 109.0 and 97.0 Various other A small peaks are identified including a 189.1 m/z peak, but these other peaks are not discussed by Tiller.

As the Examiner acknowledges, Tiller et al. does not teach to use of HTLC with MS/MS. Thus, this limitation has not been met with respect to all of the pending claims. Various additional deficiencies in the teachings of Tiller et al. not acknowledged by the examiner are identified.

Claim 2: It cannot be stated that Tiller et al. teach to isolate an ion with a mass/charge ratio (m/z) of about 289.1 ± 0.5 and effect a collision with an inert collision gas to produce either the 109.2 ± 0.5 or 96.9 ± 0.5 daughter ions. Tiller et al. does not mention the m/z 298 ion other than to label it in a chromatogram.

Claims 14-16: Tiller et al. does not mention any inert collision gas.

Claim 19: Tiller et al. does not teach or suggest in HTLC/MS/MS deproteinating a human test sample prior to ionization.

Claim 20: Tiller et al. does not teach or suggest in HTLC/MS/MS deproteinating with formic acid a test sample that is blood, serum, plasma, or urine.

Claims 23 and 35: Tiller et al. does not teach SELDI/MS/MS/TOF mass spectroscopy. Tiller et al. uses atmospheric pressure chemical ionization (APCI) (page 121, right column).

Claims 24 and 36: Tiller et al. does not teach APPI/MS/MS/TOF mass spectroscopy. APPI refers to atmospheric pressure photoionization. Tiller et al. uses atmospheric pressure chemical ionization (APCI) (page 121, right column).

Claims 26 and 38: Tiller et al. does not teach a reference sample 2, 2, 4, 6, 6-d5 testosterone.

Claims 27 and 39: Tiller et al. does not teach 2, 2, 4, 6, 6-d5 testosterone detection using ions having a mass/charge ratio 294.1 ± 0.5, 113.2 ± 0.5, or 99.9 ± 0.5.

Claims 28 and 40: Tiller et al. does not teach 2, 2, 4, 6, 6-d5 testosterone detection by generating a precursor ion having a mass/charge ratio (m/z) of about 294.1 ± 0.5 and therefrom following isolation, generating a daughter ion having a mass/charge ratio 294.1 ± 0.5, 113.2 ± 0.5, or 99.9 ± 0.5, one of which is detected.

Claims 29 and 41: Tiller et al. does not teach determining the specificity of testosterone detection by calculating a ratio of the daughter ions for the sample and comparing that ratio with a daughter ion ratio for a purified sample of testosterone.

Claims 30 and 42: Tiller et al. does not teach to detect testosterone by combining the signal for ion m/z 109.2 ± 0.5 with the signal for ion m/z 96.9 ± 0.5 to obtained a summed daughter ion signal.

Claims 31 and 43: Tiller et al. does not teach to detect testosterone by combining the signal for ion m/z 109.2 ± 0.5 with the signal for ion m/z 96.9 ± 0.5 to obtained a summed daughter ion signal which is then compared to the summed daughter ion signal with a standard curve of summed daughter ion signals for known amounts of testosterone.

Claims 32 and 44: Tiller et al. does not teach to detect reference testosterone ion(s) by mass spectrometry by combining the signal for ion 113.2 ± 0.5, with the signal for ion and 99.9 ± 0.5 to obtained a summed daughter ion signal.

The Zimmer et al. Reference

Zimmer et al. compared HTLC- MS/MS versus solid-phase extraction LC-MS/MS versus liquid-phase extraction LC-MS/MS in the single case of two drugs, named A and B. This is essentially an academic exercise because Zimmer et al. withholds the identity and chemical nature of these two drugs "for commercial reasons." Zimmer et al., page 24, right column. Although Zimmer et al. describes that drugs A and B are 99% protein bound in plasma, it is not known whether these drugs are even related to steroids, let alone related to testosterone. Zimmer also does not teach that protein binding is the key to successfully replacing LC with HTLC for MS/MS. Thus, there is no specific motivation provided by Zimmer et al. to replace LC in the LC-MS/MS method of Tiller et al. with HTLC as taught by Zimmer et al. In fact, Tiller et al. is counter to such motivation because it teaches sample analysis times of 1-2 min, "resulting in a very high sample throughput." Tiller et al. pages 121/122.

No reasonable expectation of success also exists in the combination of Zimmer et al. with that of Tiller et al. because Zimmer et al. does not identify the nature of drugs A and B and does not teach that HTLC will replace LC in the LC-MS/MS method of Tiller et al. Furthermore, various other deficiencies in the claims, not acknowledged by the Examiner are set forth below.

Claim 2: Zimmer et al. does not cure the deficiencies in the teachings of Tiller et al. with respect to isolating an ion with a mass/charge ratio (m/z) of about 289.1 ± 0.5 and effect a collision with an inert collision gas to produce either the 109.2 ± 0.5 or 96.9 ± 0.5 daughter ions. Tiller et al. does not mention the m/z 298 ion other than to label it in a chromatogram.

Claim 5: Zimmer et al. fails to teach a purifying step that comprises: (i) applying the sample to an HTLC extraction column; (ii) washing the HTLC extraction column under conditions whereby testosterone is retained by the column; (iii) eluting retained testosterone from

the HTLC extraction column; (iv) applying the retained material to an analytical column; and (v) eluting purified testosterone from the analytical column.

Claim 6. Zimmer et al. fails to teach use of an C-12 extraction column, and a C-18 analytical column together with MS/MS.

Claim 7: Zimmer et al. fails to teach a an HTLC extraction column which comprises particles of about 50 μm, and a C-18 analytical column which comprises particles of about 4 μm used together with MS/MS.

Claim 8. Zimmer et al. fails to teach as in claim 5 wherein steps (i)-(v) are performed in an in-line automated fashion.

Claim 9: Zimmer et al. fails to teach as in claim 8 wherein steps (b) and (c) are performed in an in-line automated fashion with steps (i)-(v).

Claims 14-16: Zimmer et al. does not cure the deficiency in Tiller et al. as to the teaching of specific inert collision gases.

Claim 19: Zimmer et al. does not cure the deficiency in Tiller et al. as to the teaching of deproteinating a human test sample prior to ionization in HTLC/MS/MS.

Claim 20: Zimmer et al. does not cure the deficiency in Tiller et al. as to the teaching of deproteinating with formic acid a test sample that is blood, serum, plasma, or urine in HTLC/MS/MS.

Claims 23 and 35: Zimmer et al. does not cure the deficiency in Tiller et al. as to teaching SELDI/MS/MS/TOF mass spectroscopy. Zimmer et al. uses APCI or truboionspray (page 25, left column).

Claims 24 and 36: Zimmer et al. does not cure the deficiency in Tiller et al. as to teaching APPI/MS/MS/TOF mass spectroscopy. Zimmer et al. uses APCI or truboionspray (page 25, left column).

Claims 26 and 38: Zimmer et al. does not cure the deficiency in Tiller et al. as to teaching a reference sample 2, 2, 4, 6, 6-d5 testosterone.

Claims 27 and 39: Zimmer et al. does not cure the deficiency in Tiller et al. as to teaching 2, 2, 4, 6, 6-d5 testosterone detection using ions having a mass/charge ratio 294.1 ± 0.5, 113.2 ± 0.5, or 99.9 ± 0.5.

Claims 28 and 40: Zimmer et al. does not cure the deficiency in Tiller et al. as to teaching 2, 2, 4, 6, 6-d5 testosterone detection by generating a precursor ion having a mass/charge ratio (m/z) of about 294.1 ± 0.5 and therefrom following isolation, generating a daughter ion having a mass/charge ratio 294.1 ± 0.5, 113.2 ± 0.5, or 99.9 ± 0.5, one of which is detected.

Claims 29 and 41: Zimmer et al. does not cure the deficiency in Tiller et al. as to teaching to determine the specificity of testosterone detection by calculating a ratio of the daughter ions for the sample and comparing that ratio with a daughter ion ratio for a purified sample of testosterone.

Claims 30 and 42: Zimmer et al. does not cure the deficiency in Tiller et al. as to teaching to detect testosterone by combining the signal for ion m/z 109.2 ± 0.5 with the signal for ion m/z 96.9 ± 0.5 to obtained a summed daughter ion signal.

Claims 31 and 43: Zimmer et al. does not cure the deficiency in Tiller et al. as to teaching testosterone by combining the signal for ion m/z 109.2 ± 0.5 with the signal for ion m/z 96.9 ± 0.5 to obtained a summed daughter ion signal which is then compared to the summed daughter ion signal with a standard curve of summed daughter ion signals for known amounts of testosterone.

Claims 32 and 44: Zimmer et al. does not cure the deficiency in Tiller et al. as to teaching to detect reference testosterone ion(s) by mass spectrometry by combining the signal for ion 113.2 ± 0.5, with the signal for ion and 99.9 ± 0.5 to obtained a summed daughter ion signal.

The Quinn et al. Reference

The examiner turns to Quinn et al. for teaching of HTLC with a "particle size of about 4 μM and the use of an inline filter and automated injector The Examiner does not indicate which claim is being rejected over this very limited teaching. If the Examiner is referring to claim 7, this claim specifies that the HTLC extraction column comprises particles of about 50 μm, while that of the recited C-18 analytical column comprises particles of about 4 μm. Because claim 7 does not refer to the HTLC column as having particle size of about 4 μM, the alleged teaching in Quinn et al. is not relevant to this claim.

Furthermore, the Examiner does not indicate which Quinn et al. reference is being cited for the specified teaching. As best as Applicants can determine, the Examiner appears to be referring to Quinn et al. U.S. Patent no. 5,968,367 which, at column 5, line 58, describes a particle with an opening having a diameter of between 5 to 5,000 Angstroms. Firstly, 5,000 angstroms is equal to 0.5 μM (1 angstrom equals ten thousandths of a micron). Secondly, the number referred to in Quinn et al. is the diameter of the opening of the particle, not the diameter of the particle itself as alleged by the Examiner. Thus, it is respectfully submitted that the alleged teaching of Quinn et al. has been mis-cited and, in any event, is not relevant to any claim in the case. Finally, Quinn et al. does not cure any of the deficiencies noted for Tiller et al. taken alone or in combination with Zimmer et al.

The Starevic et al. Reference

The Examiner relies heavy on Starcevic to fill the gap between the primary reference, (Tiller et al.) and Zimmer et al. According to the Examiner, Starcevic et al. teach that "testosterone forms highly protein bound drugs from crude plasma" (citing to page 197 last paragraph and page 198, first paragraph).

First, it is noted that Starcevic et al. does not indicate what fraction of testosterone is protein bound ("testosterone circulates in plasma non-specifically bound to albumin, specifically bound to sex hormone binding globulin, and unbound (free))." However, even if one of ordinary skill understood Starcevic to teach that testosterone circulates largely bound to proteins in

plasma, Applicants respectfully submit that the Examiner's reliance on this statement to fill the motivation gap between Tiller et al. and Zimmer et al. is illusory. This is because one of ordinary skill does not know whether the success by Zimmer even relates to a steroid, let alone testosterone. As already mentioned, Zimmer et al. withholds the identity of compounds A and B used in the method. Furthermore, there is nothing in Zimmer to teach or suggest that protein binding is the key or even a factor to successfully replacing LC with HTLC for MS/MS. The fact that Starcevic published nearly four years after Zimmer yet chooses to detect testosterone with LC-MS/MS rather than HTLC-MS/MS if anything runs counter to a suggestion to combine Tiller et al. with Zimmer et al.

Furthermore, no prima facie rejection has been stated at least in the case of claims 2, 5, 6-9, 14, 16, 23-32 and 35-44 for the additional reason that the rejection has failed to teach or suggest all the elements of the claims as Applicants have carefully documented above in the discussion of Zimmer et al. The rejection deficient in this regard is not cured merely by asserting that these different embodiments represent conventional working conditions.

The Ropero-Miller et al. Reference

The Examiner relies on Ropero-Miller for teaching the use of deprotination methods as allegedly being routine in the art. Applicants assume that this reference is being asserted against claims 19 and 20. Ropero-Miller et al. teaches deproteination in conjunction with GC/EI/MS, which is entirely different from the HTLC/MS/MS approach of the present claims. The lack of relevance of Ropero-Miller to the present claims clearly evidences its inability to cure the many deficiencies cited for Tiller et al. Zimmer et al., Quinn et al. and Starcevic et al., taken alone or in any combination.

It is respectfully submitted that no prima facie obviousness rejection has been stated and that the Examiner is improperly using hindsight based on Applicants' teachings to combine Tiller et al. with Zimmer et al. and further with that of the other references. Accordingly, the Examiner is urged to reconsider and withdraw the obviousness rejection over Tiller et al. in view

of Zimmer et al., in view of Quinn et al., and further in view of Starcevic et al. as evidenced by Roper-Miller et al.

CONCLUSION

Applicant respectfully submits that the pending claims are in condition for allowance. An early notice to that effect is earnestly solicited. Should any matters remain outstanding, the Examiner is encouraged to contact the undersigned at the address and telephone number listed below so that they may be resolved without the need for additional action and response thereto.

Respectfully submitted,

Date June 6, 2005

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